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1 **Roscovitine exacerbates *Mycobacterium abscessus* infection**
2 **by reducing NADPH oxidase-dependent neutrophil trafficking**

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24
25 **Keywords:** Cystic fibrosis; Roscovitine; CFTR; Neutrophil; NADPH oxidase; *Mycobacterium abscessus*;
26 Inflammation; Infection; Innate immunity; Zebrafish

29 **Abstract**

30 Persistent neutrophilic inflammation associated with chronic pulmonary infection causes progressive lung
31 injury and eventually death in individuals with cystic fibrosis (CF), a genetic disease caused by bi-allelic
32 mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

33 We therefore examined whether Roscovitine, a cyclin-dependent kinase inhibitor that (in other conditions)
34 reduces inflammation while promoting host defence, might provide a beneficial effect in the context of CF.

35 Herein, using CFTR-depleted zebrafish larvae as an innovative vertebrate model of CF immuno-
36 pathophysiology, combined with murine and human approaches, we sought to determine the effects of
37 Roscovitine on innate immune responses to tissue injury and pathogens in CF condition.

38 We show that Roscovitine exerts anti-inflammatory and pro-resolution effects in neutrophilic inflammation
39 induced by infection or tail amputation in zebrafish. Roscovitine reduces overactive epithelial ROS-mediated
40 neutrophil trafficking, by reducing DUOX2/NADPH-oxidase activity, and accelerates inflammation resolution
41 by inducing neutrophil apoptosis and reverse migration. Importantly, while Roscovitine efficiently enhances
42 intracellular bacterial killing of *Mycobacterium abscessus* in human CF macrophages *ex vivo*, we found that
43 treatment with Roscovitine results in worse infection in mouse and zebrafish models. By interfering with
44 DUOX2/NADPH oxidase-dependent ROS production, Roscovitine reduces the number of neutrophils at
45 infection sites, and consequently compromises granuloma formation and maintenance, favouring
46 extracellular multiplication of *M. abscessus* and more severe infection.

47 Our findings bring important new understanding of the immune-targeted action of Roscovitine and have
48 significant therapeutic implications for safety targeting inflammation in CF.

49

50

51 Introduction

52 Cystic fibrosis (CF) is a fatal disorder resulting from mutations in the cystic fibrosis transmembrane
53 conductance regulator (CFTR)¹. The leading causes of premature death in CF individuals is progressive
54 pulmonary injury and respiratory failure caused by mucus obstruction, infections and inflammation².

55 In CF lungs, impaired CFTR results in airway surface liquid dehydration and collapse of mucociliary
56 clearance, predisposing to recurrent infections with a subsequent hyper-inflammatory profile². CF infections
57 are typified by pathogenic bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Burkholderia*
58 *cenocepacia* or the non-tuberculous mycobacteria *Mycobacterium abscessus* (Mabs)³. In addition, CFTR
59 deficiency results in abnormal activation of macrophage and epithelial cell responses to pathogens⁴,
60 releasing pro-inflammatory mediators, such as IL8 and reactive oxygen species (ROS). This favours the
61 onset of an exuberant influx of neutrophils⁴⁻⁷, which nonetheless fails to control infections and worsens lung
62 function^{8,9}. Moreover, defects in CFTR impair the ability of neutrophils to undergo apoptosis¹⁰⁻¹² and reverse
63 migration⁷ leading to increased neutrophil activity and longevity and therefore contribute to sustained
64 pulmonary inflammation^{7,12}. Evidence suggests that inflammation may even precede infection in CF
65 airways¹³⁻¹⁵. Elevated inflammatory markers in the bronchoalveolar lavage fluid of CF infants are found, even
66 in the absence of detectable infection¹⁶. In particular, we have demonstrated that CFTR dysfunction directly
67 alters the response of epithelial cells to “sterile” injury and leads to exuberant ROS production through the
68 DUOX2/NADPH oxidase, driving an overactive neutrophil response in a CFTR-depleted zebrafish model⁷.

69 Reducing the deleterious impact of inflammation is therefore an important therapeutic goal in CF¹⁷.
70 Conventional anti-inflammatory therapies in CF include the use of glucocorticoids or ibuprofen which are
71 potentially effective but associated with significant long term side effects¹⁸. CFTR modulators have been
72 shown to reduce inflammation, however their high cost and mutation/age restriction preclude widespread
73 use. Antibiotic treatment alone is insufficient to prevent inflammatory lung damage and can induce
74 antimicrobial resistance. Although inflammation is reduced with anti-inflammatory treatment¹⁹, chronic
75 inflammation remains a consistent feature, indicating a continued need for novel approaches to prevent
76 inflammation-mediated tissue destruction in CF.

77 One potential and interesting alternative is represented by Roscovitine, an inhibitor of cyclin-dependent
78 kinases (CDK)²⁰. In particular, this compound is capable of inducing neutrophil apoptosis^{21,22}, accelerating
79 the resolution of inflammation²³⁻²⁵. Importantly, Roscovitine has proven beneficial in enhancing apoptosis of
80 neutrophils isolated from CF patients¹¹. However, the pro-apoptotic activity of Roscovitine has never been
81 evaluated in *in vivo* models of CF. Roscovitine also exerts anti-inflammatory actions on macrophages^{26,27},
82 eosinophils^{28,29} and lymphocytes³⁰. Moreover, Roscovitine enhances bactericidal activity of CF alveolar
83 macrophages^{31,32}. However, Roscovitine has not been tested in CF infection models. Roscovitine is currently
84 being evaluated in a phase 2 clinical trial in CF patients infected with *P. aeruginosa*, as a potential anti-
85 pseudomonas therapy <https://clinicaltrials.gov/ct2/show/NCT02649751?term=roscovitine&rank=1>.

86 Here, we demonstrate that Roscovitine can restore normal levels of inflammation in a *in vivo* model of CF
87 by *i*) reducing epithelial ROS production-driven neutrophil mobilisation and *ii*) enhancing neutrophil apoptosis
88 and reverse migration. Importantly, beside macrophage-directed bactericidal effect of Roscovitine, we show
89 that Roscovitine promotes an increased susceptibility to Mabs infection *in vivo* by inhibiting DUOX2/NADPH

90 oxidase-dependent neutrophil trafficking. This study represents a clear demonstration of the protective role
91 of DUOX2-mediated ROS production against Mabs infection.

92

93

94

95 **Methods**

96

97 Bacterial strains, human cells, mouse and zebrafish lines and detailed methods associated with all
98 procedures below are available in **Supplemental Methodology**.

99

100 **Zebrafish experiments**

101 Zebrafish experiments were conducted according to guidelines from the UK Home Office under AWERB and
102 in compliance with the European Union guidelines for handling of laboratory animals.

103

104 **Mouse experiments**

105 Mouse procedures were authorised by Ethics Committee A783223 (APAFIS#11465-2016111417574906).

106

107 **Macrophage experiments**

108 Primary human macrophages were generated from peripheral blood samples from consented healthy and
109 individuals with CF volunteers (approved by regional ethics approval REC12/WA/0148).

110

111 **Quantification and statistical analysis**

112 Statistical analysis was performed using Prism 7.0 (GraphPad Software) and detailed in each Figure legend.
113 ns, not significant ($p \geq 0.05$); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

114

115 Results

116 117 Roscovitine rebalances early neutrophil infiltration by epithelial ROS-dependent mechanisms

118 We first proceeded to examine the potential benefits of Roscovitine in reducing neutrophilic inflammation
119 by exploiting the zebrafish model of sterile inflammation^{7,33,34}. In zebrafish larvae, tail fin amputation triggers
120 neutrophil infiltration towards wound, accurately mimicking the kinetics and fates observed in human
121 inflammatory responses^{33,35}. In particular, zebrafish neutrophils have the same function as human
122 neutrophils and respond in a similar manner to chemicals, including Roscovitine²³.

123 In order to investigate the effect of Roscovitine on neutrophilic response, we exploited the
124 *TgBAC(mpx:EGFP)i114* line harbouring green-fluorescent neutrophils³³, in normal and CFTR-deficient
125 contexts, using *cftr* morphants (*cftr* MO)⁶ or the knockout *cftr*^{sh540} mutant (*cftr* -/-)⁷. To first address whether
126 Roscovitine influences early neutrophil infiltration, injured-WT and CF larvae were incubated with
127 Roscovitine, or *i*) the NADPH-oxidase blocker Diphenyleneiodonium (DPI), known to inhibit early neutrophil
128 mobilisation^{7,36}, *ii*) the pro-resolution drug Tanshinone IIA (TIIA), which does not influence early neutrophil
129 chemotaxis^{7,37} and *iii*) DMSO. Roscovitine treatment, but not TIIA, was able to reduce neutrophil influx in WT
130 and CF injured-fish, effectively rebalancing overactive neutrophil mobilisation in CF to that of WT levels
131 (**Figures 1A-B**). Interestingly, comparative analysis showed similar wound-associated neutrophil number in
132 both DPI- and Roscovitine-treated larvae. Epithelial release of H₂O₂, through the DUOX2/NADPH oxidase, is
133 required for the early neutrophil response to injury^{7,38,39}. We then investigated the potential anti-oxidative
134 action of Roscovitine on the recruitment of early-arriving neutrophils, by measuring ROS production in
135 injured CF fish. Compared to DMSO-treated animals, microscopy revealed that Roscovitine caused a
136 substantial inhibition of epithelial ROS production, as judged by decreased CellROX fluorescence intensity at
137 the wound (**Figures 1C-D**). This finding suggests that Roscovitine modulates the earliest phase of neutrophil
138 mobilisation to injury in an epithelial oxidase-dependent manner.

139 Collectively, these results indicate that Roscovitine reduces CF-associated inflammation by reducing both
140 epithelial oxidase activity and early neutrophil influx to injured tissue in CFTR-depleted zebrafish.

141 142 Roscovitine-driven neutrophil apoptosis and reverse migration accelerate inflammation resolution *in* 143 *vivo*

144 CF zebrafish exhibit persistent neutrophilic inflammation after injury⁷. We therefore investigated whether
145 Roscovitine treatment could resolve such a response to initiate regenerative processes.

146 WT and CF *TgBAC(mpx:EGFP)i114* larvae were injured and, 4 hours later, exposed to Roscovitine or
147 DMSO. Roscovitine reduced established post-wounding neutrophilic inflammation in WT²³ and CF contexts
148 (**Figures 2A-B**). Pro-resolution events such as local neutrophil apoptosis and migration of neutrophils away
149 from inflamed sites play a critical role to reduce inflammation and restore tissue homeostasis^{37,40,41}. We first
150 examined the extent of neutrophil apoptosis *in vivo* in CF zebrafish. Combined confocal imaging and
151 quantification of TUNEL-positive neutrophils showed that CFTR-deficient larvae treated with Roscovitine
152 exhibited enhanced neutrophil apoptosis at wound at 8 hours post-amputation (hpA), compared to their
153 control counterparts (**Figures 2C-D**). Interestingly, Roscovitine induces neutrophil apoptosis more efficiently
154 than TIIA (**Supp 1A**). We then investigated whether Roscovitine could also influence neutrophil retrograde

155 migration by examining and comparing the dynamics of neutrophil reverse migration in DMSO- and
156 Roscovitine-treated larvae using *Tg(mpx:Gal4)sh267;Tg(UAS:Kaede)i222* larvae (**Figure 2E**)^{7,42,43}.
157 Remarkably, Roscovitine significantly enhanced neutrophil reverse migration in injured CF fish (**Figures 2F-**
158 **G**). However, Roscovitine is a much less potent inducer of neutrophil reverse migration than TIIA (**Supp 1B**).

159 Efficient inflammation resolution plays a pivotal role preventing tissue damage, as well as initiating tissue
160 healing and repair⁴⁴⁻⁴⁶. The pro-resolution property of Roscovitine, linked to increased neutrophil apoptosis
161 and reverse migration, prompted us to analyse tissue repair potential in zebrafish treated with Roscovitine.
162 Despite evidence of reduced damage to regenerated tissues, our results indicated that defective tissue
163 repair was not reversed by Roscovitine exposure in CF animals (**Supp 2A-B**).

164 Overall, we show that Roscovitine promotes resolution of established neutrophilic inflammation and
165 alleviates inflammatory damage in CFTR-depleted fish by enhancing both neutrophil apoptosis and reverse
166 migration.

167

168 **Roscovitine exposure compromises epithelial ROS-dependent neutrophil mobilization during Mabs** 169 **infection**

170 As neutrophils represent the first line of defence against invading bacteria, including the multi-drug
171 resistant pathogen Mabs^{47,48}, we were next interested in determining the effect of Roscovitine on neutrophil
172 responses during Mabs infection, using a zebrafish model of Mabs infection^{49,50}. Chemoattraction of
173 neutrophils was assessed by injecting Mabs expressing *tdTomato* into the somite of *TgBAC(mpx:EGFP)i114*
174 larvae as previously described⁴⁸. As shown in **Figures 3A-C**, Roscovitine exposure resulted in a significant
175 reduction in neutrophil mobilisation towards Mabs-infected tissue. Neutrophil chemotaxis is known to require
176 functional epithelial ROS signalling⁵¹, suggesting this could also account for the Mabs-induced neutrophil
177 response. While injection of Mabs consistently triggers oxidative responses in infected tissues, confocal
178 microscopy showed abnormal oxidative activity in Roscovitine-treated larvae, which causes a substantial
179 inhibition of epithelial ROS generation at the site of infection, as reflected by the decreased CellROX signal
180 (**Figures 3A-B**). Noteworthy, this reduction of ROS production coincides with a reduced number of
181 neutrophils mobilised towards bacilli in fish exposed to Roscovitine (**Figure 3A**). Additionally, confocal
182 examination of Mabs-granuloma, a protective structure improving the control of Mabs infection⁴⁸, revealed an
183 abnormal granuloma architecture in Roscovitine-treated larvae, typified by reduced neutrophil infiltration
184 (**Figure 3C**).

185 To further support zebrafish experiments, the neutrophil influx and activity were also evaluated in mice
186 infected with Mabs then treated with Roscovitine or DMSO. Neutrophil numbers in lung compartments were
187 enumerated at 6 days post-infection (dpi). As shown in **Figure 3D**, Roscovitine-treated mice exhibited
188 reduced Ly6C^{hi} / Ly6G^{hi} staining, indicating that activated neutrophil amounts has decreased in lung after
189 Roscovitine administration. Reduced relative numbers of activated neutrophil following Roscovitine treatment
190 was confirmed by comparative analysis of cell composition in lung in these mice (**Figures 3E-G**). Of note, no
191 changes in global neutrophil numbers were observed in zebrafish or mice, ensuring that the observed
192 differences did not result from Roscovitine-induced neutropenia (data not shown).

193 Together these findings indicate that Roscovitine alters neutrophil mobilisation towards Mabs, likely by
194 interfering with epithelial oxidative activity induced by Mabs infection, in addition to the critical role played in
195 granuloma integrity with deleterious consequences such as extracellular mycobacterial multiplication⁴⁸.

196 197 **Roscovitine exposure leads to exacerbation of Mabs infection *in vivo***

198 Neutrophils are dispensable for defence against Mabs infection^{48,49,52}. The profound alteration of
199 neutrophil chemotaxis to Mabs caused by Roscovitine, led us to hypothesise that Roscovitine may hamper
200 host defence against Mabs and thus increase susceptibility to Mabs infection.

201 In order to test whether Roscovitine influences Mabs infection outcomes, intracellular Mabs killing was
202 firstly investigated *ex vivo*, using primary macrophages obtained from both healthy and CF volunteers
203 (**Figure 4A**). Relative luminescent units (RLU) analysis revealed a lower bacterial load in Mabs-infected
204 macrophages treated with Roscovitine compared to vehicle alone at 24 hpi (**Figure 4B**), suggesting that
205 Roscovitine can enhance macrophage Mabs killing in the context of CF. Interestingly, as previously reported,
206 this might depend on the acidification of macrophages³², since Roscovitine improves acidification of
207 macrophage lysosomes post Mabs infection, as shown by enhanced lysosomal fusion with intracellular Mabs
208 and increased acidified lysosome numbers in macrophages (**Supp 3A-D**). To exclude direct Roscovitine-
209 induced Mabs killing as the cause of enhanced mycobacterial clearance in macrophages, we evaluated
210 minimum inhibitory concentrations. None of the Mabs variants showed direct Roscovitine susceptibility
211 (**Table 1**), indicating that this compound has no direct antibacterial activity against Mabs. We demonstrate
212 here that Roscovitine enhances macrophage-mediated intracellular killing of Mabs, likely by improving the
213 lysosomal acidification in macrophages. However, little is known about the effect of Roscovitine on bacterial
214 control *in vivo*.

215 Next, to establish whether Roscovitine treatment could affect the control of Mabs infection *in vivo*,
216 zebrafish larvae were intravenously infected with Mabs⁵⁰ (**Figure 4A**). Our results indicated that both control-
217 and *cflr*-MO exposed to Roscovitine displayed hyper-susceptibility to Mabs, correlating with increased larval
218 mortality (**Figure 4C**) and higher bacterial loads (**Figure 4D**). Furthermore, microscopy observations showed
219 that the increase in bacterial loads in Roscovitine-treated fish correlates with replicating extracellular
220 bacteria, translating into increased number of abscesses and cord in the central nervous system of larvae⁴⁹
221 (**Figure 4D**). This is consistent with a reduced host defence and representative of severe Mabs infection in
222 zebrafish⁴⁸, and thus supports the hypothesis that Roscovitine treatment impedes the control of Mabs.
223 Importantly, a similar impact of Roscovitine upon bacterial load was observed in mice infected with Mabs.
224 Indeed, infected mice treated with Roscovitine displayed reduced ability to clear Mabs (**Figure 4E**) in the first
225 days of infection, likely due to reduced neutrophil activity (**Figures 3D-F**). These phenotypes are in line with
226 the increased bacterial loads in Roscovitine-treated mice infected with *Streptococcus pneumoniae*⁵³.

227 Collectively, these results indicate that despite the favourable impact of Roscovitine on macrophage-
228 mediated killing of Mabs, its activity increases *in vivo* susceptibility to Mabs infection, likely by hampering
229 neutrophil chemotaxis towards infected sites and the nascent granuloma.

230 231 **DUOX2/NADPH-oxidase-driven neutrophil recruitment is crucial to control of Mabs *in vivo***

232 Release of H₂O₂ gradients by epithelial cells through DUOX2/NADPH oxidase has been implicated in
233 neutrophil chemotaxis to infected tissues⁵¹. Our results above suggest that epithelial ROS generation is
234 required for neutrophil mobilization in response to Mabs infection (**Figure 3A**). We therefore investigated
235 whether DUOX2 activity drives neutrophil recruitment to Mabs infection sites. DUOX2/NADPH oxidase was
236 depleted⁵⁴ and the dynamic of neutrophils recruitment examined in *TgBAC(mpx:EGFP)i114* larvae.
237 Inactivation of NADPH oxidase activity through injection of the *duox2* morpholino impaired neutrophil
238 mobilization to the Mabs-infected somite (**Figures 5A-B**). This implies that DUOX2/NADPH oxidase-
239 dependent ROS production is specifically required for early neutrophil chemotaxis towards Mabs.
240 Additionally, confocal imaging underscored reduced number of neutrophil-associated granuloma in the
241 absence of *duox2* signalling (**Figure 5C**). Importantly, loss of DUOX2 correlated with a defective neutrophil
242 trafficking phenotype and abnormal granuloma architecture, similar to the one observed in infected fish
243 treated with Roscovitine (**Figures 3B-C**). To characterise the role of *duox2* in Mabs infection control, both R
244 and S variants were intravenously injected into control- and *duox2*-MO embryos. *duox2* knockdown resulted
245 in a higher susceptibility to Mabs infections, associated with increased larval killing (**Figures 5D-G**) and
246 enhanced bacterial loads, as demonstrated by determination of the fluorescent pixel count (FPC; **Figures**
247 **5E-H**) and whole-larvae imaging (**Figures 5F-I**), further substantiating the importance of DUOX2/NADPH
248 oxidase in controlling Mabs infection. Importantly, the increased susceptibility to Mabs infections in absence
249 of DUOX2 activity correlates with enhanced extracellular bacterial multiplication, as evidenced by the higher
250 number of abscesses (**Figures 5J-K**) as well as altered granuloma integrity (**Figures 5L-M**).

251 Together, these results indicate that release of DUOX2/NADPH oxidase-dependent ROS production at
252 the infected sites represents a critical host defence against Mabs and demonstrate that the DUOX2 axis-
253 dependent attraction of neutrophils is instrumental to efficiently contain bacteria within homeostatic
254 granulomas, thereby preventing extracellular mycobacterial spread and limiting subsequent acute infection
255 and larval mortality.

256

257 Discussion

258 Overactive neutrophil activity has been directly correlated with the onset of bronchiectasis and airway
259 damage in CF, which in term causes lung function impairment and eventually death of people with CF. Thus,
260 reducing the impact of neutrophil inflammation-mediated lung damage is a major concern in CF.

261 Among the attractive and innovative molecules to target pathways that are specific of the CF lung
262 pathophysiology, Roscovitine shows multiple beneficial proprieties. In particular, Roscovitine stimulates
263 macrophage bactericidal activity³² and promotes neutrophil apoptosis¹¹ *ex vivo* in models of CF, suggesting
264 that Roscovitine might simultaneously enhance bacterial killing and promote inflammation resolution,
265 therefore prevent subsequent infectious and inflammatory lung damage in CF. However, evaluating
266 Roscovitine in a CF animal model of infection or inflammation was awaited.

267 Here, moving from *ex vivo* through *in vivo* models of infection or inflammation, in both normal and CF
268 conditions, we sought to determine the effect of Roscovitine on neutrophilic inflammation and how its activity
269 influences the outcomes of infection and inflammation. Our findings indicate that Roscovitine exerts anti-
270 inflammatory and pro-resolution effects in neutrophil response elicited by either Mabs infection or sterile
271 injury. The proposed mechanism by which Roscovitine influences neutrophil trafficking suggests a reduced
272 epithelial ROS burden due to its inhibiting property on DUOX2/NADPH-oxidase.

273 Whereas previous studies did not investigate Roscovitine effects early after induction of inflammation, our
274 results reveal that Roscovitine especially attenuated neutrophil mobilisation rapidly after infection or injury.
275 Importantly, our findings show that diminished neutrophil response coincided with a reduced epithelial
276 oxidative activity in CF zebrafish treated with Roscovitine. This concurs with described reduced ROS
277 production after Roscovitine treatment in a carrageenan-induced pleurisyin mouse model of inflammation⁵⁵.
278 Several mechanisms could be proposed to explain the action of Roscovitine on epithelial oxidative response,
279 including a down-regulation of calcium release⁵⁶, NF- κ B²⁶ or TNF α ⁵⁵ expression, as well as direct inhibition of
280 DUOX2/NADPH-oxidase. Neutrophil mobilisation being predominantly elicited by DUOX2-mediated epithelial
281 H₂O₂^{36,57}, our data suggest that by rebalancing epithelial ROS production, Roscovitine could be able to
282 regulate early neutrophil mobilisation towards infected or inflamed tissue in CF.

283 Neutrophil apoptosis is impaired in CF^{7,58} and can be reversed by Roscovitine in CF patient-derived
284 neutrophils¹¹. Furthermore, here we show that Roscovitine is able to induce *in vivo* apoptosis in CF zebrafish
285 neutrophils. This study represents the first demonstration of the pro-apoptotic action of Roscovitine on
286 neutrophils in an *in vivo* model of inflammation in the context of CFTR deficiency. CF-related inflammation is
287 also determined by alterations in neutrophil reverse migration *in vivo*⁷. Reverse migration of neutrophil plays
288 a crucial role in the resolution of inflammation in CF, since restoring this process using TIIA significantly
289 rebalance neutrophil response in CFTR-depleted zebrafish⁷. Here we show for the first time, that Roscovitine
290 can acts on CF zebrafish to restore the reverse migration ability of neutrophils, uncovering a new potential
291 therapeutic mechanism for Roscovitine to drive inflammation resolution in CF. The mechanisms by which
292 Roscovitine influences neutrophil reverse migration is particularly intriguing and deserve further attention.

293 CF zebrafish show impaired tissue regeneration after tail-fin amputation⁷, in part due to an unresolved
294 neutrophilic inflammation, and which can be restored by pharmacological manipulation of neutrophil
295 responses using TIIA⁷. Interestingly, while Roscovitine profoundly alleviates neutrophilic inflammation, our
296 experiments show that Roscovitine does not improve tissue repair in injured fish. Possible explanations for

297 this finding include the following: (i) Roscovitine inhibits proteins CDK²⁰ and p38MAKP⁵⁹, as well as epithelial
298 ROS production : all these pathways are pivotal in the activation of regenerative processes; (ii) blocking
299 CDK9 using Roscovitine delayed macrophage recruitment to injury⁶⁰, an important cell population in the
300 processes of tissue repair⁶¹; (iii) in contrast to TIIA, Roscovitine preferentially directs the neutrophil towards
301 apoptosis rather than reverse migration. Following Roscovitine treatment, the large amount of apoptotic
302 neutrophils generated could interfere with the efferocytosis potential of macrophages and thus might exert a
303 prolonged local pro-inflammatory state delaying tissue repair.

304 With the slow development of new treatments and since Roscovitine is readily available and well-tolerated⁶²,
305 these findings could have significant therapeutic implications for potently targeting inflammation in CF lung
306 disease, and thus may support currently therapeutic strategies or could be an alternative to existing anti-
307 inflammatory approaches. These data also suggest Roscovitine might have beneficial effects on the pancreas
308 destruction and CF-related diabetes⁶³ or gastrointestinal and colorectal cancers in CF^{64,65}. While CF is
309 principally characterised by pulmonary infection and inflammation, intestinal disruption involving chronic
310 inflammation is also a frequent feature⁶⁴. In CF, epithelial surfaces produce an increased ROS burden⁷ with
311 potential genotoxic consequences. While ROS are directly mutagenic to DNA, H₂O₂ produced in epithelia is
312 a potent chemoattractant source for neutrophils, driving local inflammation³⁶, itself a known driver of
313 tumourigenesis⁶⁶. Moreover, ROS production is also a proliferative signal in many epithelial cell types⁶⁷.
314 Interestingly, Duox2 knockout significantly alleviate intestinal inflammation in a mouse model of ileocolitis⁶⁸,
315 suggesting that targeting DUOX2-mediated ROS production might show promise in the treatment of
316 gastrointestinal cancer in people with CF. Firstly known for its anti-cancer properties, Roscovitine is currently
317 being tested in several phase I and II clinical trials against human cancers⁶⁹. So, by restoring normal level of
318 inflammation in CF, Roscovitine might also, by reducing cell proliferation, epithelial ROS-mediated
319 mutagenesis and inflammation, prevent cancer in CF patients.

320 Mabs infections are associated with severe pneumonia and accelerated inflammatory lung damage in CF
321 patients^{70,71}. In line with results previously obtained^{31,32}, Roscovitine reduces intracellular bacterial loads in
322 both WT and CF macrophages infected with Mabs, likely by enhancing their ability to kill bacteria. As
323 intracellular bacterial destruction by professional phagocytes is crucial to control Mabs infection^{6,72}, perhaps
324 stimulating antibacterial activity using Roscovitine and thereby precluding the establishment of an acute
325 infection could be a therapeutic strategy in CF-related Mabs infection. Roscovitine stimulates macrophage
326 bactericidal activity by restoring intra-phagolysosome acidic pH^{31,32} (which is abnormally high in CF
327 macrophages⁷³). Having shown that professional phagocytes acidify phagosomes to efficiently control
328 Mabs^{72,74,75}, Roscovitine-mediated intra-phagosomal acidification could account for the Mabs infection
329 phenotype. Interestingly, Roscovitine was found to inhibit Nox2-mediated ROS production in nociceptive
330 neurons through the blockade of Cdk5⁵⁵. Nox2-mediated ROS production in macrophages and neutrophils is
331 another important antibacterial actor against Mabs⁶. These results could suggest that phagosomal
332 acidification is a more potent microbicidal mechanism against Mabs than ROS activity in phagocytes. At this
333 stage, the differential importance of acidic and oxidative defences in the control of Mabs remains to be firmly
334 established. It will be interesting to see whether Roscovitine influences oxidative responses against Mabs.
335 Answering these questions will provide evidence on the most interesting antibacterial mechanisms that could

336 be enhanced therapeutically to better deal with Mabs infections. In addition, whether Roscovitine influences
337 the antibacterial defence of neutrophils has not yet been tested and remains to be addressed.

338 Unexpectedly, while Roscovitine was able to enhance Mabs killing *ex vivo*, a substantial exacerbation of
339 Mabs infection was found in mice and zebrafish treated with Roscovitine. In particular, Mabs-infected
340 zebrafish rapidly succumbed when exposed to Roscovitine in both WT and CF conditions. Hyper-
341 susceptibility to Mabs due to the Roscovitine exposure is associated with increased extracellular Mabs
342 multiplication and abnormal granuloma maintenance which are representative of a profound impairment in
343 Mabs control^{48,49}. Importantly, this increased susceptibility to Mabs coincides with reduced neutrophil
344 mobilization and activity towards infected compartments in mouse and zebrafish. Our previous work
345 highlighted the critical role of neutrophils in the control of Mabs infection by phagocytosing and killing
346 bacilli^{47,76} and by favouring the formation of granulomas able to restrict extracellular multiplication of Mabs⁷⁷.
347 Zebrafish failed to mount a normal epithelial oxidative response to pathogens when treated with Roscovitine,
348 strongly suggesting that Roscovitine affects ROS-driven chemotaxis guiding neutrophils to the nascent
349 granulomas, potentially promoting extracellular Mabs growth and thereby an acute infection.

350 Although studies postulated that infection-associated neutrophil recruitment is dispensable to epithelial
351 ROS production⁷⁸, we demonstrate the capacity of neutrophils to migrate in DUOX2-derived ROS dependant
352 manner in response to Mabs, that would be directly involved in the formation of protective granulomas. This
353 result shows for the first time that host-derived epithelial ROS signalling, mediated by DUOX2/NADPH
354 oxidase, can prime neutrophil chemotaxis to Mabs infection and therefore defines a critical role for DUOX2
355 activity in the control of Mabs infection. As a consequence, oxidative activity blockade by Roscovitine
356 increases the risk of impeding host innate immune response and therefore promote an overwhelming Mabs
357 infection. However, since Roscovitine showed enhanced efficacy in combination with other existing
358 therapeutics such as CFTR modulators³¹, Roscovitine will likely diminish the severity of inflammatory lung
359 injury driven by microbial components, host inflammatory mediators as well as genetic defect in CFTR, and
360 accelerated recovery in the context of antibiotic therapy in CF patients.

361 In addition, while apoptosis is essential for neutrophil shutdown and initiating inflammation resolution, the
362 reduced number of neutrophils due to the pro-apoptotic Roscovitine action may also affects the ability of
363 immune system to efficiently respond to Mabs infection. In contrast, reverse-migrated neutrophils were found
364 able to mount a response to *S. aureus* infection *in vivo*⁷⁹. At this stage, the role of neutrophil reverse
365 migration in the process of infection and inflammation in CF remains to be fully characterised. Reverse
366 migration could have the potential to be deleterious, allowing localised infection or inflammation to
367 disseminate⁸⁰. Alternatively, encouraging neutrophil egress from infected or inflamed sites could serve as a
368 pro-resolving mechanism^{7,37}. Answering these questions in CF pulmonary disease will determine how best to
369 harness apoptosis or reverse migration for therapeutic purposes to drive inflammation resolution while
370 minimizing the risk of impaired innate immunity in people with CF.

371 To conclude, CFTR mutations affect mucus properties, inflammatory processes and antibacterial
372 defences. These different aspects are intertwined: treating one of these features has consequences on the
373 other two. Given its anti-oxidative action, the application of Roscovitine in CF could induce counterproductive
374 and needs therefore to be further studied.

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396

397 **Authorship Contributions**

398 AB conceived the study and wrote the manuscript with input from SAR, RAF and J-LH. RAF, J-LH and AB
399 designed experiments and analysed data. RAF, J-LH and AB guided and supervised the work. CL provided
400 zebrafish tools. VLM, DR-R, SG, C-MD, AAAS and AB performed experiments. All authors contributed to the
401 article and approved the submitted version.

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404 **Disclosure of Conflicts of Interest**

405 The authors declare that the research was conducted in the absence of any commercial or financial
406 relationships that could be construed as a potential conflict of interest.

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409 **References**

- 410 1. Gadsby DC, Vergani P, Csanády L. The ABC protein turned chloride channel whose failure causes
411 cystic fibrosis. *Nature*. 2006;440(7083):477–483.
- 412 2. Elborn JS. Cystic fibrosis. *Lancet*. 2016;388(10059):2519–2531.
- 413 3. Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clin. Microbiol. Rev.*
414 2002;15(2):194–222.
- 415 4. Döring G, Gulbins E. Cystic fibrosis and innate immunity: How chloride channel mutations provoke

- 416 lung disease. *Cell. Microbiol.* 2009;11(2):208–216.
- 417 5. Cantin AM, Hartl D, Konstan MW, Chmiel JF. Inflammation in cystic fibrosis lung disease:
418 Pathogenesis and therapy. *J. Cyst. Fibros.* 2015;14(4):419–430.
- 419 6. Bernut A, Dupont C, Ogryzko N V., et al. CFTR Protects against Mycobacterium abscessus Infection
420 by Fine-Tuning Host Oxidative Defenses. *Cell Rep.* 2019;26(7):1828-1840.e4.
- 421 7. Bernut A, Loynes CA, Floto RA, Renshaw SA. Deletion of cfr Leads to an Excessive Neutrophilic
422 Response and Defective Tissue Repair in a Zebrafish Model of Sterile Inflammation. *Front. Immunol.*
423 2020;11:.
- 424 8. Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. *Thorax.* 2009;64(1):81–88.
- 425 9. McCarthy C, Emmet O'Brien M, Pohl K, Reeves EP, McElvaney NG. Airway inflammation in cystic
426 fibrosis. *Eur. Respir. Monogr.* 2014;64:14–31.
- 427 10. Dibbert B, Weber M, Nikolaizik WH, et al. Cytokine-mediated Bax deficiency and consequent delayed
428 neutrophil apoptosis: A general mechanism to accumulate effector cells in inflammation. *Proc. Natl.*
429 *Acad. Sci. U. S. A.* 1999;96(23):.
- 430 11. Moriceau S, Lenoir G, Witko-Sarsat V. In cystic fibrosis homozygotes and heterozygotes, neutrophil
431 apoptosis is delayed and modulated by diamide or roscovitine: Evidence for an innate neutrophil
432 disturbance. *J. Innate Immun.* 2010;2(3):260–266.
- 433 12. Gray RD, Hardisty G, Regan KH, et al. Delayed neutrophil apoptosis enhances NET formation in
434 cystic fibrosis. *Thorax.* 2018;73(2):134–144.
- 435 13. Khan TZ, Wagener JS, Bost T, et al. Early pulmonary inflammation in infants with cystic fibrosis. *Am.*
436 *J. Respir. Crit. Care Med.* 1995;151(4):1075–1082.
- 437 14. Armstrong DS, Grimwood K, Carlin JB, et al. Lower airway inflammation in infants and young children
438 with cystic fibrosis. *Am. J. Respir. Crit. Care Med.* 1997;
- 439 15. Corvol H, Fitting C, Chadelat K, et al. Distinct cytokine production by lung and blood neutrophils from
440 children with cystic fibrosis. *Am. J. Physiol. - Lung Cell. Mol. Physiol.* 2003;284(6 28-6):.
- 441 16. Verhaeghe C, Delbecq K, de Leval L, Oury C, Bours V. Early inflammation in the airways of a
442 cystic fibrosis foetus. *J. Cyst. Fibros.* 2007;6(4):304–308.
- 443 17. Mitri C, Xu Z, Bardin P, et al. Novel Anti-Inflammatory Approaches for Cystic Fibrosis Lung Disease:
444 Identification of Molecular Targets and Design of Innovative Therapies. *Front. Pharmacol.* 2020;11:.
- 445 18. Lands LC, Stanojevic S. Oral non-steroidal anti-inflammatory drug therapy for lung disease in cystic
446 fibrosis. *Cochrane Database Syst. Rev.* 2019;2019(9):.
- 447 19. Hisert KB, Heltshe SL, Pope C, et al. Restoring cystic fibrosis transmembrane conductance regulator
448 function reduces airway bacteria and inflammation in people with cystic fibrosis and chronic lung
449 infections. *Am. J. Respir. Crit. Care Med.* 2017;195(12):1617–1628.
- 450 20. Meijer L, Borgne A, Mulner O, et al. Biochemical and cellular effects of roscovitine, a potent and
451 selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. *Eur. J. Biochem.*
452 1997;243(1–2):527–536.
- 453 21. Rossi AG, Sawatzky DA, Walker A, et al. Cyclin-dependent kinase inhibitors enhance the resolution
454 of inflammation by promoting inflammatory cell apoptosis. *Nat. Med.* 2006;12(9):1056–1064.
- 455 22. Leitch AE, Riley NA, Sheldrake TA, et al. The cyclin-dependent kinase inhibitor R-roscovitine down-

- 456 regulates Mcl-1 to override pro-inflammatory signalling and drive neutrophil apoptosis. *Eur. J.*
457 *Immunol.* 2010;40(4):.
- 458 23. Robertson JD, Ward JR, Avila-Olias M, Battaglia G, Renshaw SA. Targeting Neutrophilic
459 Inflammation Using Polymersome-Mediated Cellular Delivery. *J. Immunol.* 2017;198(9):3596–3604.
- 460 24. Koedel U, Frankenberg T, Kirschnek S, et al. Apoptosis is essential for neutrophil functional shutdown
461 and determines tissue damage in experimental pneumococcal meningitis. *PLoS Pathog.* 2009;5(5):.
- 462 25. Cartwright JA, Lucas CD, Rossi AG. Inflammation resolution and the induction of granulocyte
463 apoptosis by cyclin-dependent kinase inhibitor drugs. *Front. Pharmacol.* 2019;10(FEB):
- 464 26. Jhou RS, Sun KH, Sun GH, et al. Inhibition of cyclin-dependent kinases by olomoucine and
465 roscovitine reduces lipopolysaccharide-induced inflammatory responses via down-regulation of
466 nuclear factor κ b. *Cell Prolif.* 2009;42(2):141–149.
- 467 27. Du J, Wei N, Guan T, et al. Inhibition of CDKS by roscovitine suppressed LPS-induced \bullet NO
468 production through inhibiting NF κ B activation and BH4 biosynthesis in macrophages. *Am. J. Physiol. -*
469 *Cell Physiol.* 2009;297(3):.
- 470 28. Farahi N, Uller L, Juss JK, et al. Effects of the cyclin-dependent kinase inhibitor R-roscovitine on
471 eosinophil survival and clearance. *Clin. Exp. Allergy.* 2011;41(5):673–687.
- 472 29. Duffin R, Leitch AE, Sheldrake TA, et al. The CDK inhibitor, R-roscovitine, promotes eosinophil
473 apoptosis by down-regulation of Mcl-1. *FEBS Lett.* 2009;583(15):.
- 474 30. Yoshida H, Kotani H, Kondo T, et al. CDK inhibitors suppress Th17 and promote iTreg differentiation,
475 and ameliorate experimental autoimmune encephalomyelitis in mice. *Biochem. Biophys. Res.*
476 *Commun.* 2013;435(3):.
- 477 31. Shrestha CL, Zhang S, Wisniewski B, et al. (R)-Roscovitine and CFTR modulators enhance killing of
478 multi-drug resistant Burkholderia cenocepacia by cystic fibrosis macrophages. *Sci. Rep.* 2020;10(1):.
- 479 32. Riazanski V, Gabdoulkhakova AG, Boynton LS, et al. TRPC6 channel translocation into phagosomal
480 membrane augments phagosomal function. *Proc. Natl. Acad. Sci. U. S. A.* 2015;112(47):E6486–
481 E6495.
- 482 33. Renshaw SA, Loynes CA, Trushell DMI, et al. Atransgenic zebrafish model of neutrophilic
483 inflammation. *Blood.* 2006;108(13):3976–3978.
- 484 34. Renshaw SA, Loynes CA, Elworthy S, Ingham PW, Whyte MKB. Modeling inflammation in the
485 Zebrafish: How a fish can help us understand lung disease. *Exp. Lung Res.* 2007;33(10):549–554.
- 486 35. Henry KM, Loynes CA, Whyte MKB, Renshaw SA. Zebrafish as a model for the study of neutrophil
487 biology. *J. Leukoc. Biol.* 2013;94(4):633–642.
- 488 36. Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide
489 mediates rapid wound detection in zebrafish. *Nature.* 2009;459(7249):996–9.
- 490 37. Robertson AL, Holmes GR, Bojarczuk AN, et al. A zebrafish compound screen reveals modulation of
491 neutrophil reverse migration as an anti-inflammatory mechanism. *Sci. Transl. Med.* 2014;6(225):.
- 492 38. Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide
493 mediates rapid wound detection in zebrafish. *Nature.* 2009;459(7249):996–999.
- 494 39. Gamaley IA, Klyubin I V. Roles of reactive oxygen species: Signaling and regulation of cellular
495 functions. *Int. Rev. Cytol.* 1999;188:203–255.

- 496 40. Buckley CD, Gilroy DW, Serhan CN, Stockinger B, Tak PP. The resolution of inflammation. *Nat. Rev.*
497 *Immunol.* 2013;13(1):59–66.
- 498 41. De Oliveira S, Rosowski EE, Huttenlocher A. Neutrophil migration in infection and wound repair:
499 Going forward in reverse. *Nat. Rev. Immunol.* 2016;16(6):378–391.
- 500 42. Elks PM, Van Eeden FJ, Dixon G, et al. Activation of hypoxia-inducible factor-1 α (hif-1 α) delays
501 inflammation resolution by reducing neutrophil apoptosis and reverse migration in a zebrafish
502 inflammation model. *Blood.* 2011;118(3):712–722.
- 503 43. Holmes GR, Anderson SR, Dixon G, et al. Repelled from the wound, or randomly dispersed? Reverse
504 migration behaviour of neutrophils characterized by dynamic modelling. *J. R. Soc. Interface.*
505 2012;9(77):3229–3239.
- 506 44. Karin M, Clevers H. Reparative inflammation takes charge of tissue regeneration. *Nature.*
507 2016;529(7586):307–315.
- 508 45. Mescher AL, Neff AW, King MW. Inflammation and immunity in organ regeneration. *Dev. Comp.*
509 *Immunol.* 2017;66:98–110.
- 510 46. Eming SA, Wynn TA, Martin P. Inflammation and metabolism in tissue repair and regeneration.
511 *Science (80-.).* 2017;356(6342):1026–1030.
- 512 47. Malcolm KC, Caceres SM, Pohl K, et al. Neutrophil killing of Mycobacterium abscessus by intra- and
513 extracellular mechanisms. *PLoS One.* 2018;13(4):.
- 514 48. Bernut A, Nguyen-Chi M, Halloum I, et al. Mycobacterium abscessus-Induced Granuloma Formation
515 Is Strictly Dependent on TNF Signaling and Neutrophil Trafficking. *PLoS Pathog.* 2016;12(11):.
- 516 49. Bernut A, Herrmann JL, Kissa K, et al. Mycobacterium abscessus cording prevents phagocytosis and
517 promotes abscess formation. *Proc. Natl. Acad. Sci. U. S. A.* 2014;111(10):.
- 518 50. Bernut A, Dupont C, Sahuquet A, et al. Deciphering and imaging pathogenesis and cording of
519 Mycobacterium abscessus in zebrafish embryos. *J. Vis. Exp.* 2015;2015(103):.
- 520 51. Brothers KM, Gratacap RL, Barker SE, et al. NADPH Oxidase-Driven Phagocyte Recruitment
521 Controls Candida albicans Filamentous Growth and Prevents Mortality. *PLoS Pathog.* 2013;9(10):.
- 522 52. Malcolm KC, Caceres SM, Pohl K, et al. Neutrophil killing of Mycobacterium abscessus by intra- and
523 extracellular mechanisms. *PLoS One.* 2018;13(4):.
- 524 53. Hoogendijk AJ, Roelofs JJTH, Duitman JW, et al. R-roscovitine reduces lung inflammation induced by
525 Lipoteichoic acid and Streptococcus pneumoniae. *Mol. Med.* 2012;
- 526 54. Huang C, Niethammer P. Tissue Damage Signaling Is a Prerequisite for Protective Neutrophil
527 Recruitment to Microbial Infection in Zebrafish. *Immunity.* 2018;48(5):1006-1013.e6.
- 528 55. Sandoval R, Lazcano P, Ferrari F, et al. TNF- α increases production of reactive oxygen species
529 through Cdk5 activation in nociceptive neurons. *Front. Physiol.* 2018;9(FEB):
- 530 56. Tamma G, Ranieri M, Di Mise A, et al. Effect of roscovitine on intracellular calcium dynamics:
531 Differential enantioselective responses. *Mol. Pharm.* 2013;10(12):.
- 532 57. Galli F, Battistoni A, Gambari R, et al. Oxidative stress and antioxidant therapy in cystic fibrosis.
533 *Biochim. Biophys. Acta - Mol. Basis Dis.* 2012;1822(5):690–713.
- 534 58. McKeon DJ, Condliffe AM, Cowburn AS, et al. Prolonged survival of neutrophils from patients with
535 Δ F508 CFTR mutations. *Thorax.* 2008;63(7):660–661.

- 536 59. Kim J-E, Park H, Choi S-H, Kong M-J, Kang T-C. Roscovitine Attenuates Microglia Activation and
537 Monocyte Infiltration via p38 MAPK Inhibition in the Rat Frontoparietal Cortex Following Status
538 Epilepticus. *Cells*. 2019;8(7):746.
- 539 60. Hoodless LJ, Lucas CD, Duffin R, et al. Genetic and pharmacological inhibition of CDK9 drives
540 neutrophil apoptosis to resolve inflammation in zebrafish in vivo. *Sci. Rep.* 2016;5:.
- 541 61. Nguyen-Chi M, Laplace-Builhé B, Travnickova J, et al. TNF signaling and macrophages govern fin
542 regeneration in zebrafish larvae. *Cell Death Dis.* 2017;8(8):e2979.
- 543 62. Leven C, Schutz S, Audrezet MP, et al. Non-linear pharmacokinetics of oral roscovitine (Seliciclib) in
544 cystic fibrosis patients chronically infected with pseudomonas aeruginosa: A study on population
545 pharmacokinetics with monte carlo simulations. *Pharmaceutics*. 2020;12(11):.
- 546 63. J E. Symposium Summaries. *Pediatr. Pulmonol.* 2018;53(S2):S36–S147.
- 547 64. Abraham JM, Taylor CJ. Cystic Fibrosis & disorders of the large intestine: DIOS, constipation, and
548 colorectal cancer. *J. Cyst. Fibros.* 2017;16:S40–S49.
- 549 65. Starr TK, Allaei R, Silverstein KAT, et al. A transposon-based genetic screen in mice identifies genes
550 altered in colorectal cancer. *Science (80-)*. 2009;
- 551 66. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646–674.
- 552 67. Pinto MCX, Kihara AH, Goulart VAM, et al. Calcium signaling and cell proliferation. *Cell. Signal.*
553 2015;27(11):2139–2149.
- 554 68. Chu FF, Esworthy RS, Doroshov JH, et al. Deficiency in Duox2 activity alleviates ileitis in GPx1- and
555 GPx2-knockout mice without affecting apoptosis incidence in the crypt epithelium. *Redox Biol.*
556 2017;11:.
- 557 69. Cicenias J, Kalyan K, Sorokinas A, et al. Roscovitine in cancer and other diseases. *Ann. Transl. Med.*
558 2015;3(10):.
- 559 70. Catherinot E, Roux AL, Macheras E, et al. Acute respiratory failure involving an R variant of
560 mycobacterium abscessus. *J. Clin. Microbiol.* 2009;47(1):271–274.
- 561 71. Esther CR, Esserman DA, Gilligan P, Kerr A, Noone PG. Chronic Mycobacterium abscessus infection
562 and lung function decline in cystic fibrosis. *J. Cyst. Fibros.* 2010;9(2):117–123.
- 563 72. Roux AL, Viljoen A, Bah A, et al. The distinct fate of smooth and rough Mycobacterium abscessus
564 variants inside macrophages. *Open Biol.* 2016;6(11):.
- 565 73. Di A, Brown ME, Deriy L V., et al. CFTR regulates phagosome acidification in macrophages and
566 alters bactericidal activity. *Nat. Cell Biol.* 2006;8(9):933–944.
- 567 74. Laencina L, Dubois V, Le Moigne V, et al. Identification of genes required for Mycobacterium
568 abscessus growth in vivo with a prominent role of the ESX-4 locus. *Proc. Natl. Acad. Sci. U. S. A.*
569 2018;115(5):E1002–E1011.
- 570 75. Dubois V, Viljoen A, Laencina L, et al. MmpL8MAB controls Mycobacterium abscessus virulence and
571 production of a previously unknown glycolipid family. *Proc. Natl. Acad. Sci. U. S. A.*
572 2018;115(43):E10147–E10156.
- 573 76. Bernut A, Le Moigne V, Lesne T, et al. In Vivo assessment of drug efficacy against Mycobacterium
574 abscessus using the embryonic zebrafish test system. *Antimicrob. Agents Chemother.*
575 2014;58(7):4054–4063.

- 576 77. Bernut A, Nguyen-Chi M, Halloum I, et al. Mycobacterium abscessus-Induced Granuloma Formation
577 Is Strictly Dependent on TNF Signaling and Neutrophil Trafficking. *PLoS Pathog.* 2016;12(11):.
578 78. Deng Q, Harvie EA, Huttenlocher A. Distinct signalling mechanisms mediate neutrophil attraction to
579 bacterial infection and tissue injury. *Cell. Microbiol.* 2012;14(4):517–528.
580 79. Ellett F, Elks PM, Robertson AL, Ogryzko N V., Renshaw SA. Defining the phenotype of neutrophils
581 following reverse migration in zebrafish. *J. Leukoc. Biol.* 2015;98(6):975–981.
582 80. Colom B, Bodkin J V., Beyrau M, et al. Leukotriene B4-Neutrophil Elastase Axis Drives Neutrophil
583 Reverse Transendothelial Cell Migration InVivo. *Immunity.* 2015;42(6):.
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585

586 Figure legends

587

588 **Figure 1. Roscovitine-reduced epithelial oxidative activity rebalances early neutrophil mobilisation at** 589 **wound in CF zebrafish model**

590 (A-B) WT, *cfr*^{-/-} and *cfr* MO *TgBAC(mpx:EGFP)*i*114* larvae were pre-treated with of Roscovitine, DPI (as
591 positive control) TIIA (as negative control) or DMSO (as mock control) prior to tail fin amputation procedure,
592 then injured and immediately put back in treatments for 4 h. Neutrophil number at the wound (dotted lines)
593 was observed and enumerated at 4 hpA under a fluorescence microscope. (A) Neutrophil recruitment assay
594 (*n*= 21, Two-Way ANOVA with Dunnett's post-test, error bars represent SEM). (B) Representative number of
595 neutrophils at wound in Roscovitine- versus DMSO-treated *cfr* MO zebrafish (Scale bars, 200 μm). (C-D) *cfr*
596 MO stained with CellROX[®] to label H₂O₂ generation. Means ± SEM ROS intensity (C) and associated
597 pseudocolored photomicrographs (D) of injured tails revealing oxidative activity at 30 min post-amputation
598 (mpA) in *cfr* MO treated with Roscovitine (*n* = 12, Mann Whitney test; Scale bars, 200 μm).
599

600

601 **Figure 2. Roscovitine accelerates inflammation resolution *in vivo* both by inducing neutrophil** 602 **apoptosis and reverse migration**

603 (A-B) Control-Mo or *cfr*-MO *TgBAC(mpx:EGFP)*i*114* were injured and treated from 4 hpA with Roscovitine or
604 of TIIA. (A) Neutrophil number at the wound was observed and counted at 8 hpA (*n*=21, Two-Way ANOVA
605 with Tukey's multiples comparison test). (B) Representative number of neutrophils remaining at wounds
606 (Scale bars, 200 μm). (C-D) injured-*cfr* MO larvae were treated with Roscovitine from 4 hpA and stained
607 with TUNEL/TSA to label apoptotic cells (C) Neutrophil apoptosis quantification at 8 hpA (*n*= 15, Fisher *t*-
608 test). (D) Representative confocal pictures of injured tails (Scale bars, 50 μm) revealing the proportion of
609 apoptotic neutrophils at the wound at 8 hpA. (E-F) Reverse-migration in *cfr* MO
610 *Tg(mpx:gal4)sh267;Tg(UASkaede)*i*222* after Roscovitine treatment. At 4 hpA, neutrophils at site of injury
611 were photoconverted then the numbers of photoconverted cells (red) that migrate away (white dotted box)
612 from the photoconverted area (blue dotted box) were time-lapse imaged and quantified over 4 hours by
613 confocal microscopy (E). (F) Plot showing the number of photoconverted neutrophils leaving the wound over
614 4 hours post photoconversion (hpc). Line of best fit shown is calculated by linear regression. *P*-value shown
615 is for the difference between the 2 slopes (*n*= 12, performed as 3 independent experiments). (G)
616 Representative confocal imaging of injured tails showing the kinetics of photoconverted neutrophils that

616 move away from the area of injury over inflammation resolution.

617

618 **Figure 3. Roscovitine impedes neutrophil trafficking during Mabs infection**

619 (A-B) WT *Tg(mpx:eGFP)i114* larvae were treated with Roscovitine or DMSO then infected into the somite
620 with ≈ 100 Mabs R expressing dtTomato. Infected larvae are stained with CellROX[®] to label ROS production.

621 Representative epithelial oxidative response (arrow) and number of neutrophils at infection site in
622 Roscovitine- versus DMSO-treated larvae at 3 hours post-infection (hpi) (Scale bars, 75 μm). (B) Means \pm
623 SEM ROS intensity at the site of infection (2 hpi, $n = 8$, student t test). (C) Confocal images showing the
624 representative repartition of neutrophil-associated Mabs granuloma in larvae treated with Roscovitine
625 compared with DMSO-exposed animals (Scale bars, 10 μm).

626 (D-G) Mice were intravenously infected with R Mabs then treated with 50 μM Roscovitine or DMSO at 1dpi.
627 At 6 dpi neutrophils are isolated from the lung of mice and analysed by flow cytometry. (D) Representative
628 dot-plots showing the expression of Ly6C^{hi} / Ly6G^{hi} (activated neutrophil) among neutrophils. Graphs showing
629 the mean \pm SEM absolute (E) and relative (F) number of activated neutrophils, and related ratio of activated
630 neutrophils (G) in lungs ($n=5$, unpaired Student's *t* test, representative of 3 independent experiments).

631

632 **Figure 4. Roscovitine exacerbates Mabs infection in zebrafish and mouse model of infection**

633 (A) The effect of Roscovitine on Mabs infection outcomes was evaluated in primary human macrophage (B),
634 zebrafish (C-D) and mouse (C) model of infection. (B) Monocyte-derived primary human macrophages were
635 infected at a MOI 1:1 with bioluminescent Mabs (Mabs-lux) for 2 hours. Extracellular bacteria were washed
636 off and fresh media containing Roscovitine or DMSO added. At each specified time-point, cells were lysed
637 and viable intracellular bacteria quantified as relative luminescent units (RLU). Roscovitine enhances
638 intracellular Mabs killing in macrophages obtained from both healthy volunteers and CF patients (One-Way
639 ANOVA with Dunnett's post-test). (C-D) Control MO or *cftr* MO were intravenously infected with ≈ 100 -150
640 Mabs R expressing *tdTomato*. From one day post-infection (dpi) larvae were treated with Roscovitine or
641 DMSO. (C) Survival analysis of Control MO (left) or *cftr* MO zebrafish (right). Data are plotted as percentage
642 of surviving animals over a 10 days period ($n=30$, Mantel-Cox Log-rank test, average of two independent
643 experiments). (D) Representative whole-larvae imaging of Control MO (left) or *cftr* MO zebrafish (right) at 3
644 dpi (Scale bars, 200 μm). (E) Mice were intravenously infected with Mabs then treated 24 hours later with 10
645 and 50 μM Roscovitine or DMSO. The surviving bacteria were enumerated after 6 dpi by CFU analysis.
646 Results are expressed as \log_{10} units of CFU per organ at 1 (before treatment administration) and 6 dpi (Two-
647 Way ANOVA with Dunnett's post-test).

648

649 **Figure 5. Epithelial oxidative response-dependent recruited neutrophils restricts Mabs infection**

650 (A-B) Control MO or *duox2* MO *Tg(mpx:eGFP)i114* larvae were infected into the somite with ≈ 100 CFU Mabs
651 S expressing *dtTomato*. (A) Mean \pm SEM number of neutrophils mobilized to the infection site at 3 hpi ($n =$
652 20, average of two independent experiments) and (B) representative neutrophil-associated site of infection
653 (Scale bars, 75 μm). (C-M) Control MO or *duox2* MO were intravenously infected with ≈ 100 -150 CFU of
654 Mabs R or S expressing *tdTomato*. (C) Confocal images showing the representative repartition of neutrophil-
655 associated *Mabs* granuloma in Control MO versus *duox2* MO (Scale bars, 10 μm). (D and G) Survival

656 analysis of R- (D) or S-infected larvae (G). Data are plotted as percentage of surviving animals over a 10
657 days period (n=60, Mantel-Cox Log-rank test, Average of three independent experiments). (E and H) Mean
658 fluorescent pixel counts (FPC) of 3 dpi larvae infected by either R (E) or S (H) variants. Results are
659 expressed as log₁₀ units of FPC per fish. (F and I) Representative images of R- (F) or S-infected larvae (I) at
660 3 dpi (Scale bars, 200 μm). (J and K) Percentage of 3 dpi infected larvae with abscess (J) from three
661 independent experiments (n=30) and associated mean ± SEM number of abscess per infected animal (K).
662 (L-M) Kinetic of granuloma formation in whole embryos over a 4-day infection period (L) from three
663 independent experiments (n=30) and associated mean ± SEM number of granuloma per infected animal (M).
664 Statistical significance: Mantel-Cox Log-rank test (D and G), two-tailed unpaired Student's t test (B, E, H and
665 K), Fisher's exact test of a contingency table (J and L) or Two-Way ANOVA with Tukey's multiples
666 comparison test (M).

667

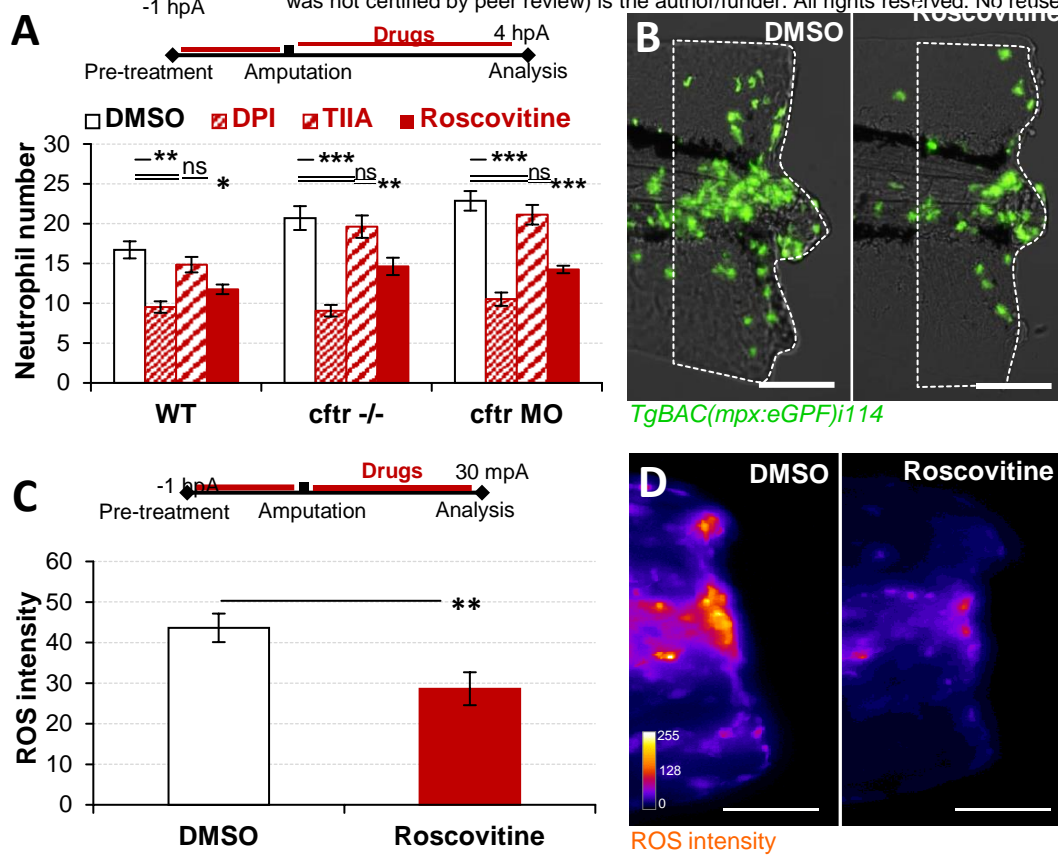


Fig1

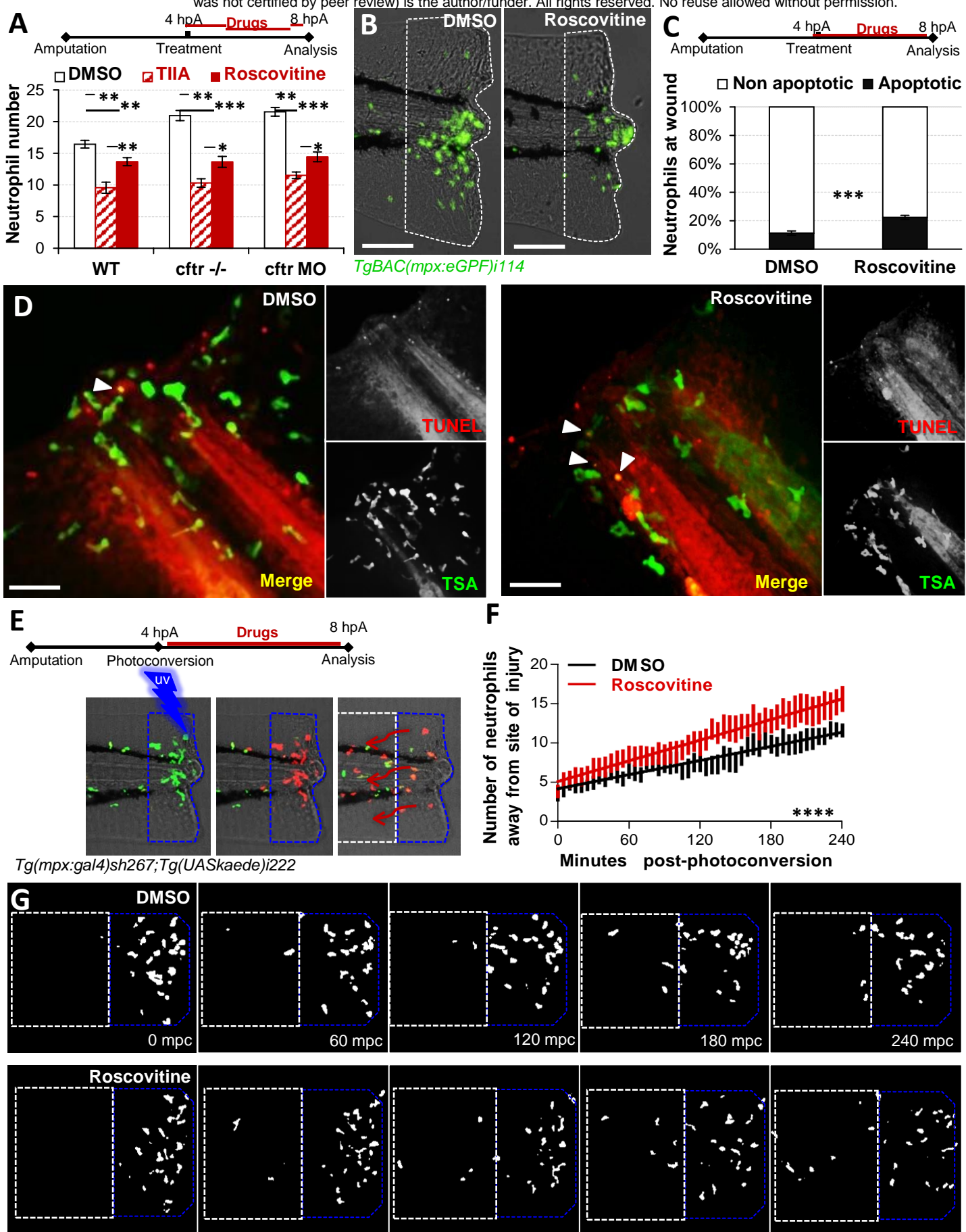


Fig2

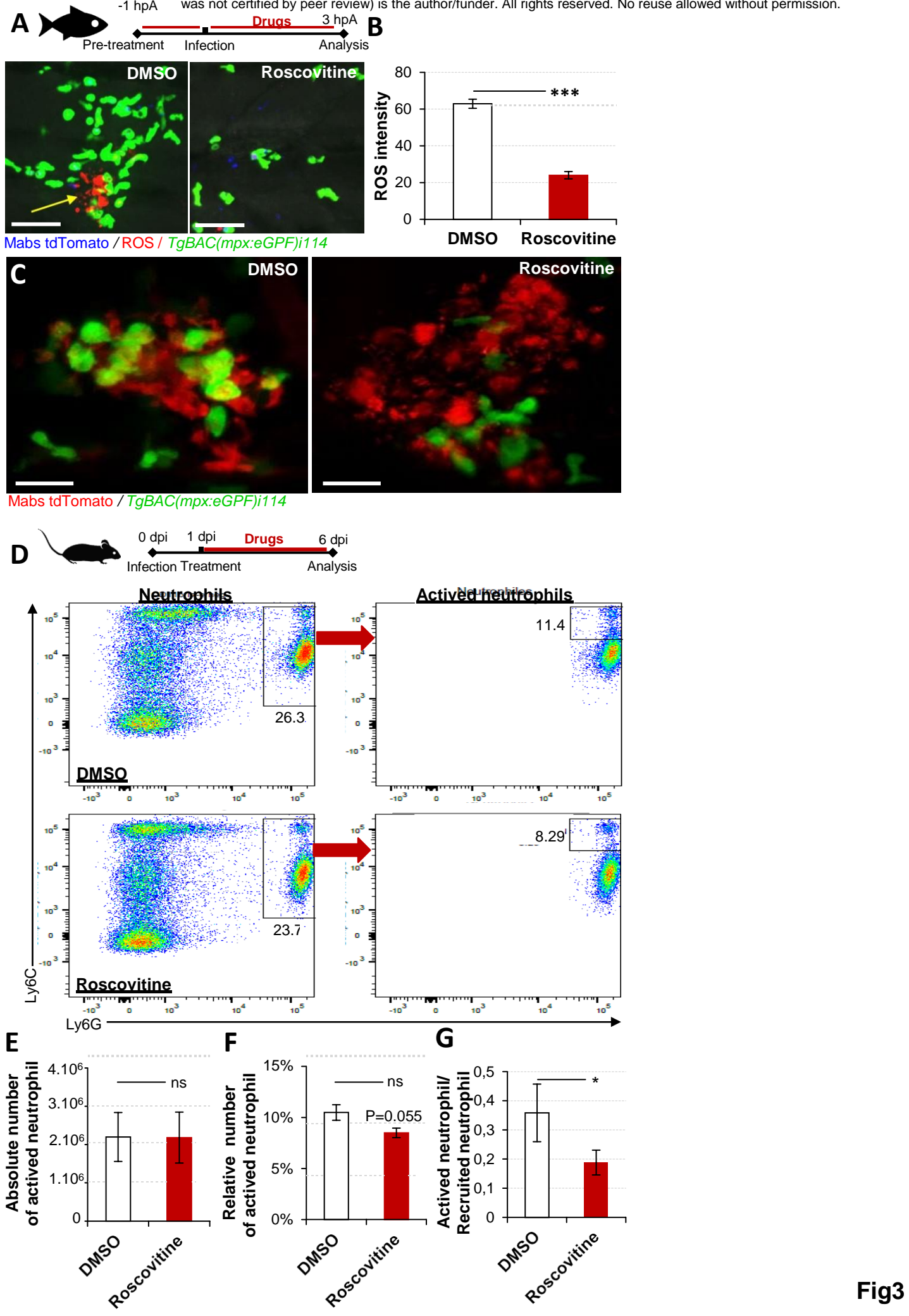


Fig3

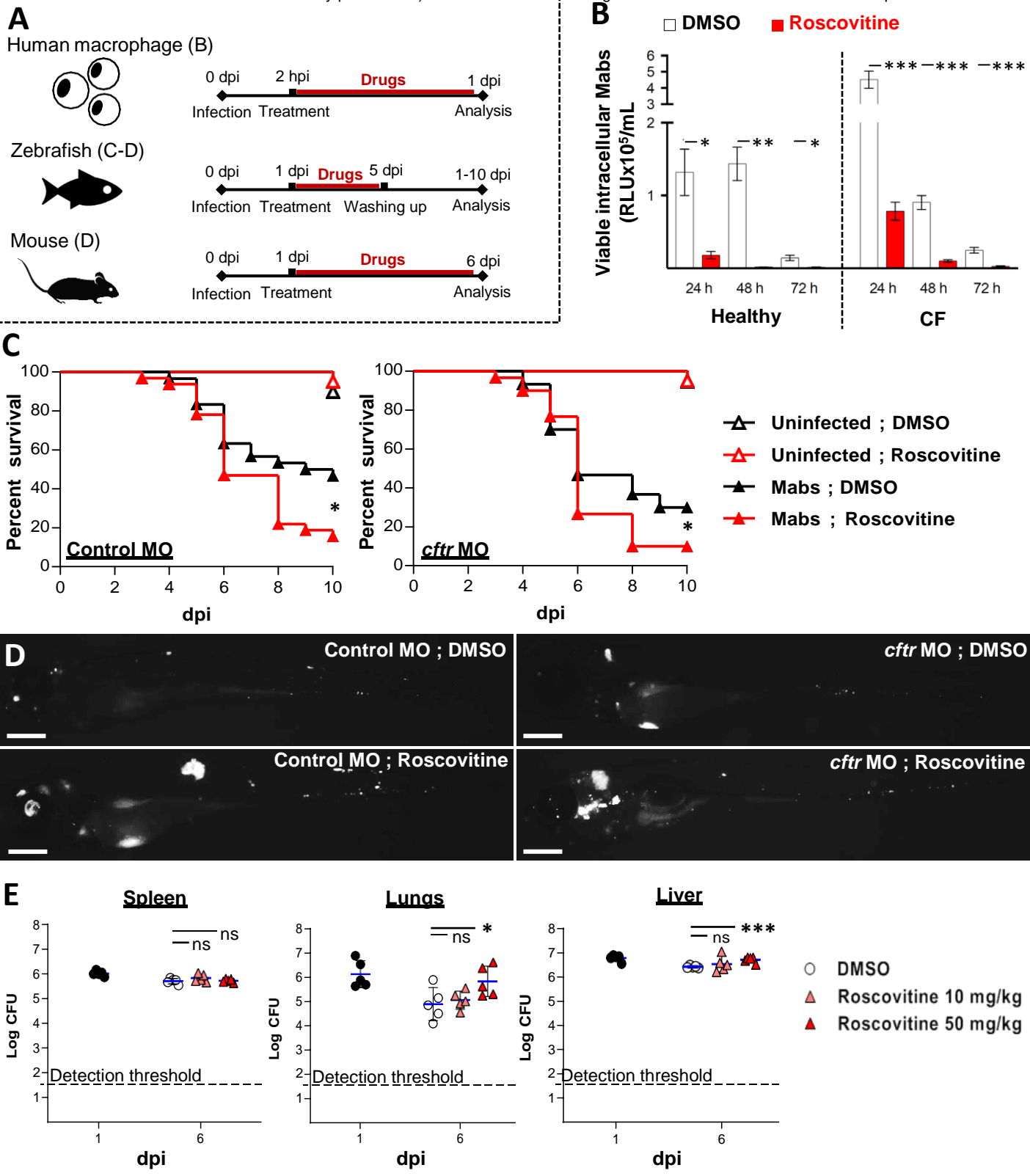


Fig4

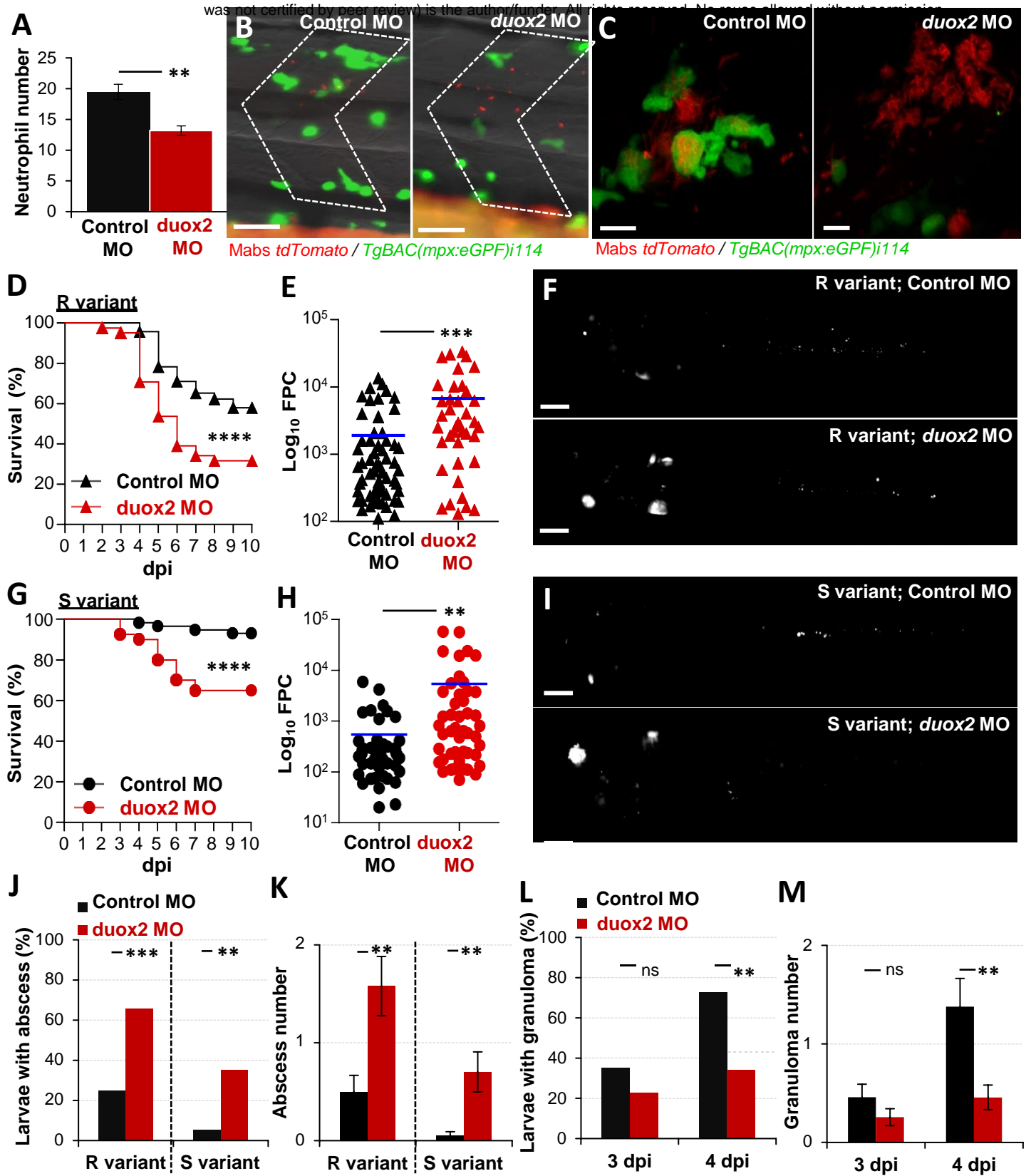


Fig5